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Morphologic Pattern of Tenascin as a Diagnostic Biomarker in Colon Cancer

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Background: Immunohistochemical methods were used to study the pattern of expression of tenascin (TN) in invasive colon cancer and its relation to prognosis.
Methods: Sixty patients (29 males, 31 females) with a mean age of 77 years were studied. TN expression was evaluated by immunohistochemistry using paraffin-embedded tissue sections, TN expression levels were correlated with patient age, tumor stage, and survival.
Results: TN positivity varied from trace to 4+. Staining patterns were as follows: in well-differentiated cancer, TN fibers form thick bands around invading tumor glands. In poorly differentiated cancer, TN fibers had an interstitial pattern surrounding individual tumor cells. Using Cox's proportional hazard regression method, survival was significantly related to TN score ($P < 0.0001$) and stage of disease ($P < 0.05$). No significant relationship was found between survival and age ($P = 0.375$).
Conclusion: Patients with more TN expression had better long-term survival than patients with no or weak TN expression. Pathologic and clinical entities in colon cancer have distinct immunohistochemical TN matrix patterns that may correlate with predictive value and long-term survival. J. Surg. Oncol. 64:98–101.

KEY WORDS: glycoprotein; extracellular matrix; adhesion molecule

INTRODUCTION

Tenascin (TN) is an extracellular matrix protein with a dynamically changing tissue distribution. In fetal life it is transiently expressed in connective tissues and in the mesenchyme of many developing epithelial organs and is often re-expressed in the stroma of malignant epithelial tumors. It is also present in the central and peripheral nervous systems. The distinctive and highly regulated expression of TN has provoked interest in identification of its possible functions in cell-cell and cell-substratum adhesion, cell migration, cell growth, and cell differentiation during morphogenesis [1]. In the normal adult colon, TN is detected at very low levels. In colonic neoplasms, TN is predominantly localized in the fibrous stroma surrounding the neoplastic glandular epithelia [2,3].

Invasion of the basement membrane, a continuous structural barrier separating epithelial tissues from adjacent stroma, is a critical stage in the complex multistep process of metastasis. Major components of the extracellular matrix include laminin, fibronectin, type IV collagen, entactin, tenascin, and heparan sulfate proteoglycans.

It is believed that mechanisms controlling tumor invasion involve multiple functional and morphological events that include tumor cell adherence to basement membrane via specific cell surface receptors, secretion of matrix degrading enzymes (collagenases), and migration

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of cells through the stroma into the circulatory and lymphatic system [4].

The relationship between tumor invasion and TN expression is controversial [5,6]. The exact role of host stromal response in metastasis is poorly understood [7]. TN production may play a major role in the desmoplastic response to invading cancer cells [1,4]. In this study, we have used an immunoperoxidase method with routinely processed formalin-fixed, paraffin-embedded surgically resected tissue to investigate the relationship between TN staining in invasive colon cancer and prognosis.

MATERIALS AND METHODS

The records of 60 patients with histologically proven adenocarcinoma of the colon entered into the Tumor Registry of the Montefiore Medical Center (MMC) between 1988 and 1990, were obtained. The clinical information supplied included the patient's date of birth, admission date to the Registry, primary tumor site (e.g., ascending, transverse, descending, sigmoid colon, or rectum), tumor grade, stage (local, regional, or distant disease), most recent follow-up (including whether the patient was alive or dead), and the initial treatment (surgery, chemotherapy, or radiation). Information on deceased patients was limited to the date of death and did not include the cause of death. For the purpose of the statistical analysis, each stage of the disease was assigned a number: local—1, regional—2, and distant—3. All of the patients underwent surgical resection of their tumors and had paraffin-embedded specimens stored at MMC.

TN was detected by immunohistochemical methods. The specimen blocks were retrieved, and three specimens were used as controls for localization and specificity of TN staining (normal colon mucosa, adenomatous polyp, invasive colon cancer). All specimens were cut into 5 mm sections and placed on lysine-coated glass slides. Slides were deparaffinized using xylene and rehydrated with a graded ethanol series (100–70%), followed by a rinse in water, incubation in 3% hydrogen peroxide for 20 min, washed in tap water, and pretreated with protease in phosphate-buffered solution (PBS) for 30 min at room temperature. The slides were rinsed in tap water and in PBS, followed by incubation with normal horse serum for 30 min at room temperature. TN monoclonal antibody (monoclonal ascites; Gibco, Gaithersburg, MD, DCRB02;1/300,000 dilution) was added and incubated overnight in a humidified chamber. Slides were washed in PBS and biotinylated antibody was added for 30 min, followed by rinse in PBS, and addition of avidin-biotin reagent for 30 min. After an additional PBS wash, the slides were treated with DAB Chromogen (DAKO, Carpinteria, CA) for 15 min. Slides were counterstained with hematoxylin, dehydrated, and mounted with transforming glass coverslips.

Fig. 1. Prominent tenascin staining around advancing malignant glands and blood vessels. Note lack of staining around normal glands (right side) (×40).

TN positivity in tumor was graded from trace to 4+ according to the total percentage of the tumor stroma that showed TN expression, (0–25% staining) trace to 1+, (25–50% staining) 2+, (50–75% staining) 3+, (75–100% staining) 4+. Intensity and patterns of staining in different tumor grades were also noted.

The associations among TN score, stage, age, and survival were analyzed using Cox's proportional hazard regression method. The survival curves for TN value were estimated using Kaplan-Meier method. The correlation between TN expression and stage were analyzed using the Spearman correlation coefficient.

RESULTS

Expression of TN in 60 human colon carcinomas was studied by immunohistochemistry and graded on a scale from trace to 4+. Expression of TN was found to be virtually negative in normal adult colonic mucosa, whereas adjacent areas of tumor showed strong TN staining (Fig. 1). In well-differentiated tumors, TN expression was seen mainly at the level of the muscularis mucosae and the stalks of dysplastic polyps. TN showed a specific staining pattern consisting of parallel fibers forming thick and thin bands, most common in the stroma surrounding invading tumor glands (Fig. 2). In poorly differentiated tumors, TN pattern of expression was diffuse and interstitial, forming a ''fishnet'' network of fibers around individual tumor cells (Fig. 3). TN expression was also found surrounding blood vessel walls and forming bands in the muscularis propria in the vicinity of infiltrating tumor (Fig. 4). In contrast, normal areas of mucosa, blood vessels, and muscularis propria were rarely positive for TN.

The relationship between TN score and patient sur-

Fig. 2. Strong tenascin staining showing bundles of fibers around advancing malignant glands (×200).

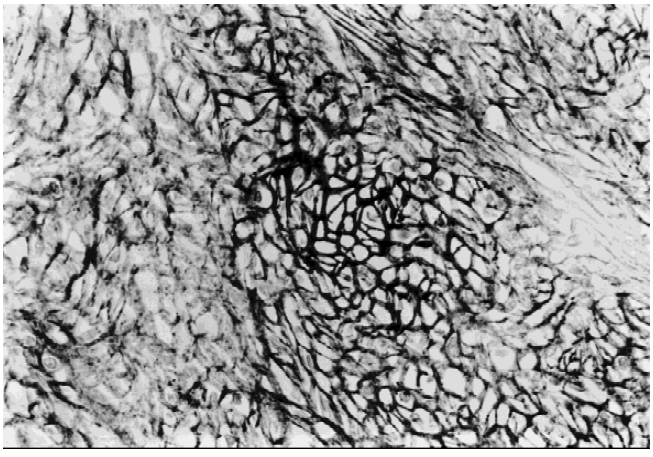

Fig. 3. Poorly differentiated adenocarcinoma with interstitial pattern of tenascin expression forming (fishnet) network around individual malignant cells (×400).

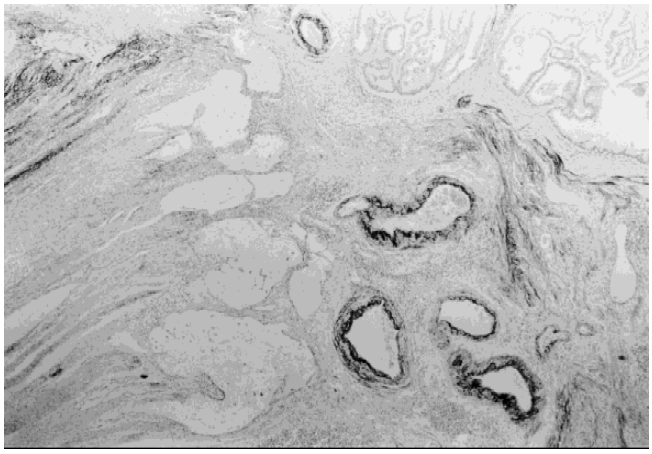

Fig. 4. Prominent tenascin staining in the wall of blood vessels in the vicinity of the tumor (×40).

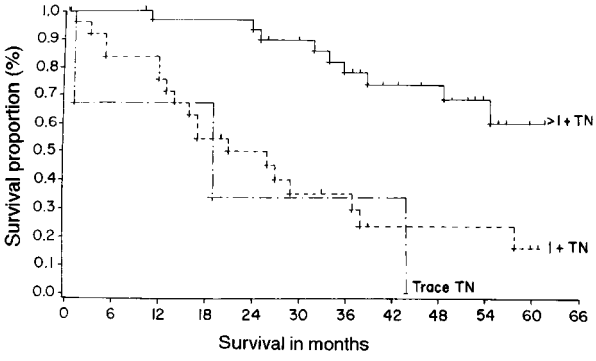

Fig. 5. The relationship between survival in months and tenascin expression, estimated by using Kaplan-Meier method.

vival was estimated by using the Kaplan-Meier method (Fig. 5), which showed that long-term survival was significantly related to TN score. The relationship among TN score, mean age, mean stage, and survival is shown in Table I. Univariate analysis, using the log rank test, showed that survival was significantly related to TN score ($P < 0.0001$) and stage of disease ($P < 0.05$). There was no significant relationship between survival and age ($P = 0.375$). Although the information we obtained from the tumor registry did not include the cause of death, and the mean age of our patients was 77 years, this analysis showed clearly that age did not play a significant role in survival and that the main factor related to survival in our patients was the amount of TN expression.

Multivariate survival analysis, using Cox's proportional hazard regression method and including both TN score and stage in the model, showed that TN was significantly related to survival ($P < 0.01$), but stage was not significantly related to survival ($P = 0.19$). For every unit increase in TN value, the estimated risk of death decreased 0.34 times and 0.41 times by univariate and multivariate analyses respectively.

TN had a trend of negative correlation with stage in that there was less TN expression in advanced stage of disease, with a Spearman correlation coefficient R of −0.22, ($P = 0.098$). No significant correlation was found between TN and age ($R = -0.06$, $P = 0.675$).

DISCUSSION

There is increasing evidence that TN plays an important role not only in embryogenesis, but also in tissue remodeling and tissue invasion in regions of tissue undergoing phenotypic changes [8]. The prominent staining of TN at cancer-mesenchymal junctions and in the walls of blood vessels in the vicinity of tumor suggests a role for TN in preventing cancer cells from invading surrounding tissues [9]. An increase in TN expression may lead to desmoplastic reaction. It has been reported that there is an inverse relationship between host stromal response and spontaneous tumor invasion and metastasis

TABLE I. Relationships Among Tenascin (TN) Score, Mean Age, Mean Stage, and Median Survival Time*

TN score	No. cases	Mean age	Mean stage	Median survival time
<1	4	69.8 ± 13.1	2.00 ± 0.8	<1 year
= 1	26	74.5 ± 10.9	2.12 ± 0.6	1 year
>1	30	74.5 ± 10.1	1.73 ± 0.6	>5 years

*Each stage was assigned a number: local disease, 1; regional disease, 2; distant disease, 3.

[3]. Existing evidence supports that the inhibition of the desmoplastic response of murine BL6 melanoma by L-3, -4 dehydroproline was found to cause increased invasion and metastasis [10]. This suggests that the desmoplastic response may have some protective value against tumor invasion and metastasis.

TN induction is caused by epithelial tumor cells that secrete factors that stimulate fibroblasts to synthesize TN in the surrounding stroma [11]. Upon staining with anti-tenascin antibodies, no intracellular staining was seen within carcinoma cells. Neither carcinoma cell lines analyzed in tissue culture [12] nor cultured carcinoma cells taken from tumor directly have been found to produce TN. Furthermore, it has been shown that the breast carcinoma cell line MCF7 can induce co-cultured fibroblasts to produce tenascin [13–15] and the secreted factor responsible for this is transforming growth factor beta [13].

Our data showed a significantly improved prognosis for patients whose tumors had intense TN staining ($P < 0.0001$). These findings are consistent with previous reports that aneuploid DNA patterns are observed at higher frequency in TN negative colon cancers and metastatic lymph nodes. In contrast, diploid DNA patterns were observed predominantly in TN positive colon cancer tissues [9].

It has been shown that tumor invasion and metastasis require enzymatic degradation of the host interstitial matrix [2]. Dissociation of the tumor cells depends on changes in the extracellular matrix (ECM) surrounding the primary tumor [16]. It also has been shown that an advancing front of invasive tumor cells can induce secretion of hydrolytic enzymes from adjacent nontumor host cells [17]. Recently it has been suggested that stromelysin-3, an enzyme that degrades ECM in cancers, plays an important part in progression of epithelial malignancy [2], and the possibility was raised that stromelysin-3 acts on TN during the invasive phase of cancer. We hypothesize that the sequence of events is as follows: (1) the presence of cancer cells in the stroma disturb the normal ECM environment, (2) the host reacts by producing TN as a remodeling support for the ECM, and (3) the invading tumor cells secrete proteolytic enzymes to dissect through the dense fibrous stroma. The degree of invasion depends on the balance between TN expression and stromal reaction and levels of proteolytic activity produced by the tumor cells. The role of TN in acting as a barrier against tumor spread may explain why tumors with decreased TN expression have worse prognosis. Although the biological functions of TN have not been clearly elucidated, TN staining in routine surgical tissue specimens may provide a useful prognostic tool to detect a subset of patients who appear to have a better prognosis.

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